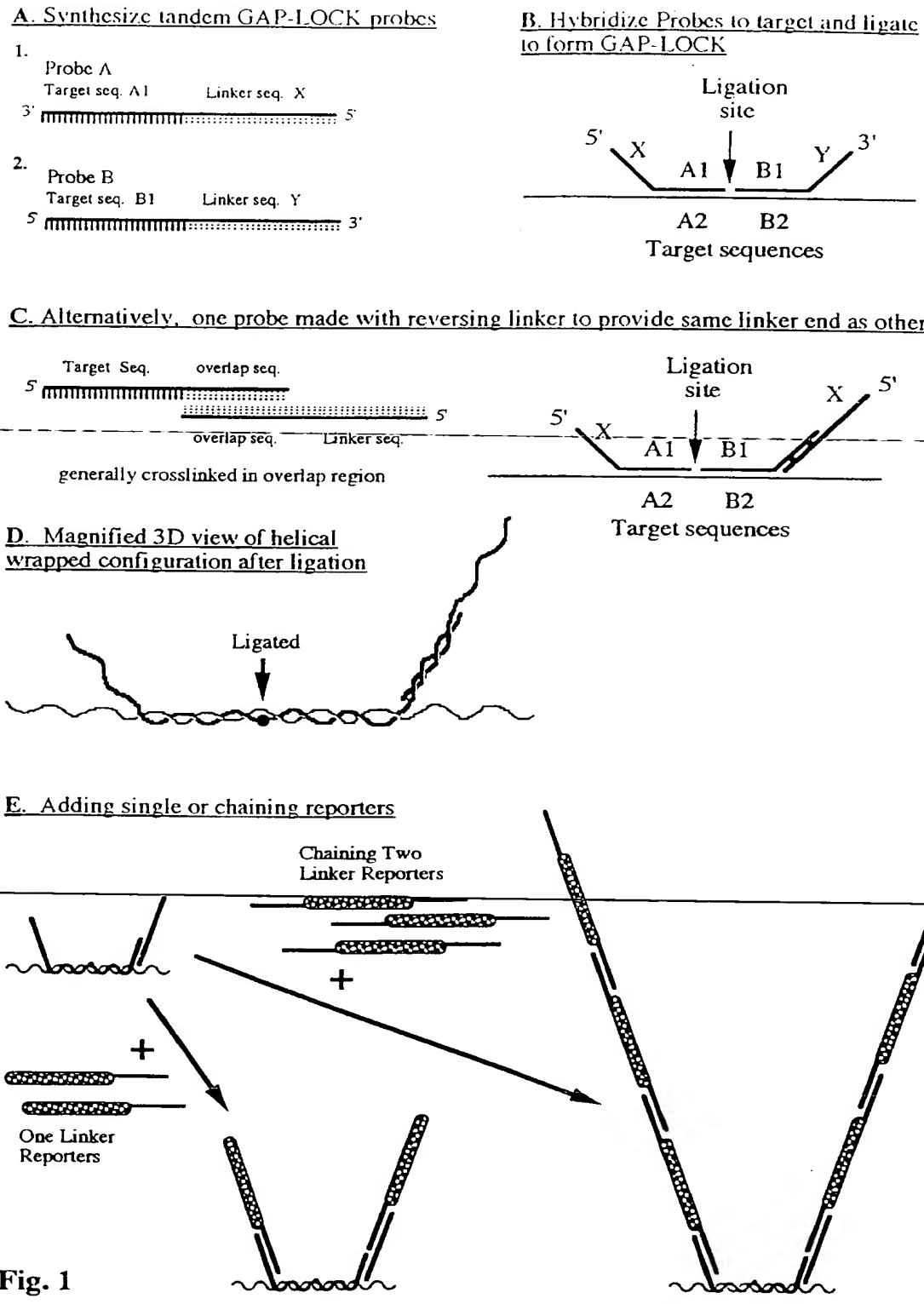
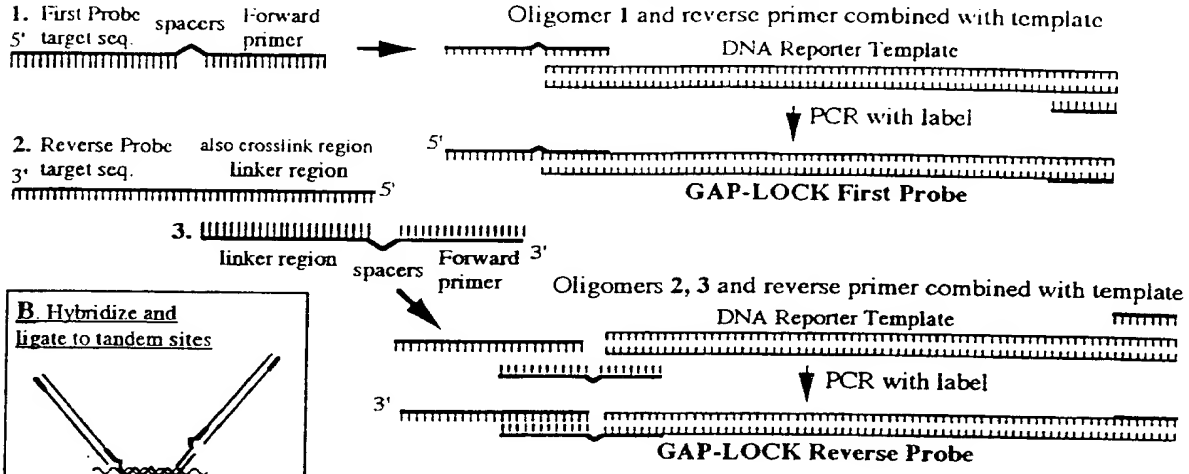

A. Synthesize tandem GAP-LOCK probes
1.
Probe A
Target seq. A1
Linker seq. X
3'
5'
2.
Probe B
Target seq. B1
Linker seq. Y
5'
3'
B. Hybridize Probes to target and ligate to form GAP-LOCK
Ligation site
5'
X
A1
B1
Y
3'
A2
B2
Target sequences
C. Alternatively, one probe made with reversing linker to provide same linker end as other probe
Target Seq.
overlap seq.
5'
5'
overlap seq.
Linker seq.
generally crosslinked in overlap region
Ligation site
5'
X
A1
B1
X
5'
A2
B2
Target sequences
D. Magnified 3D view of helical wrapped configuration after ligation
Ligated
E. Adding single or chaining reporters
Chaining Two Linker Reporters
+
+
One Linker Reporters

Fig. 1

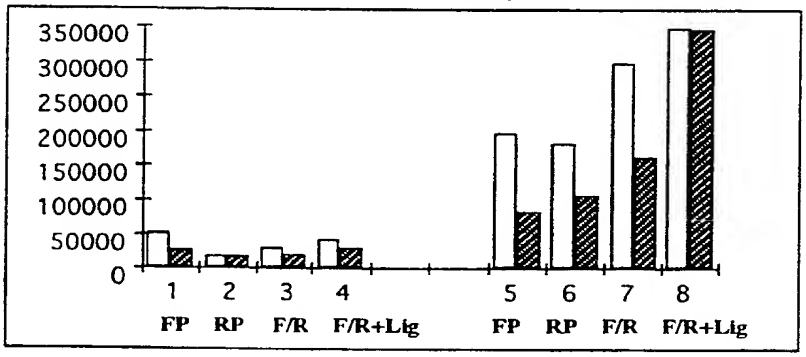
A. Alternate GAP-LOCK Method: with probes joined to reporters




B. Hybridize and ligate to tandem sites

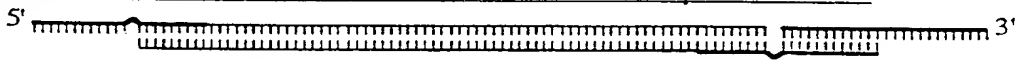


C. Dot Blots of GAP-LOCK Probes: (Example 1)




Bar 1-4 = 300 bp tails
Bar 5-8 = 800 bp tails
Open Bars = pre NaOH
Stripe Bars = post NaOH
F = First Probe
R = Reverse Probe
F/R = both probes
F/R+Lig. = ligated both probes (First and Reverse)

D. Alternate GAP-LOCK with First Probe, Reverse Probe and Reporter combined




E. GAP-LOCK with generic reporters joined to distal linkers on the First and Reverse Probes

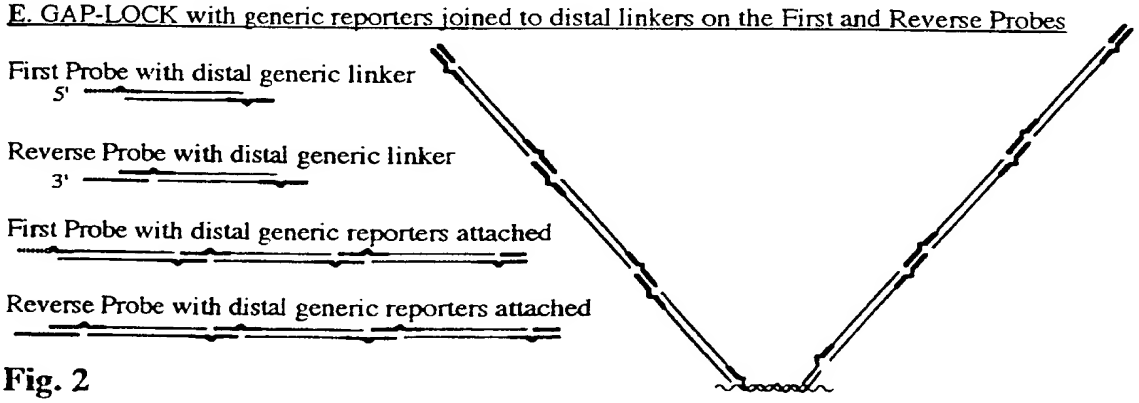



**Fig. 2**

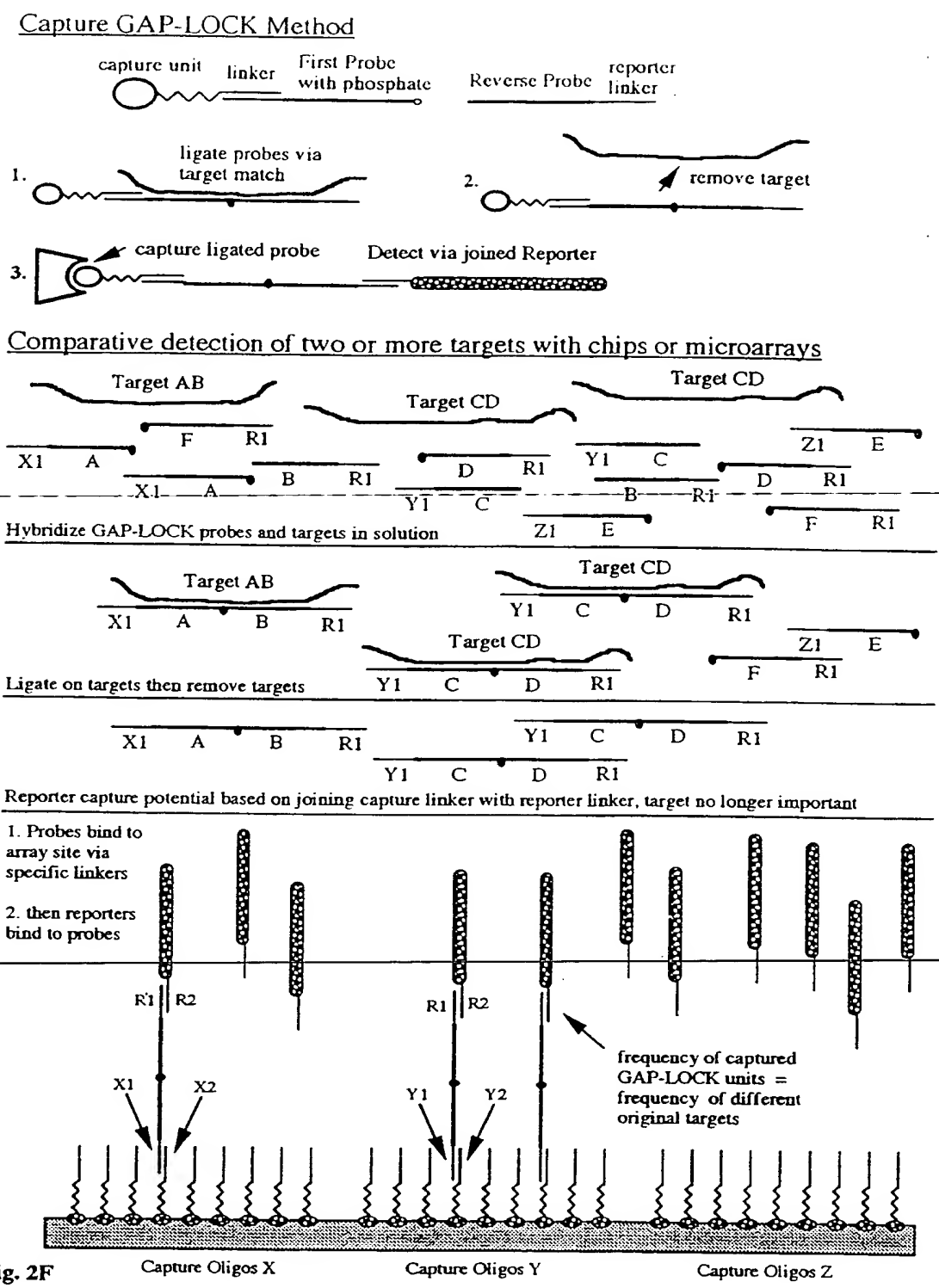
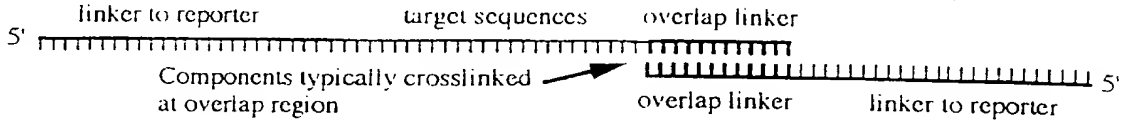

Capture GAP-LOCK Method
capture unit
linker
First Probe with phosphate
Reverse Probe
reporter linker
1.
ligate probes via target match
2.
remove target
3.
capture ligated probe
Detect via joined Reporter
Comparative detection of two or more targets with chips or microarrays
Target AB
Target CD
Target CD
Hybridize GAP-LOCK probes and targets in solution
Target AB
Target CD
Target CD
Ligate on targets then remove targets
1. Probes bind to array site via specific linkers
2. then reporters bind to probes
frequency of captured GAP-LOCK units = frequency of different original targets
Capture Oligos X
Capture Oligos Y
Capture Oligos Z

Fig. 2F

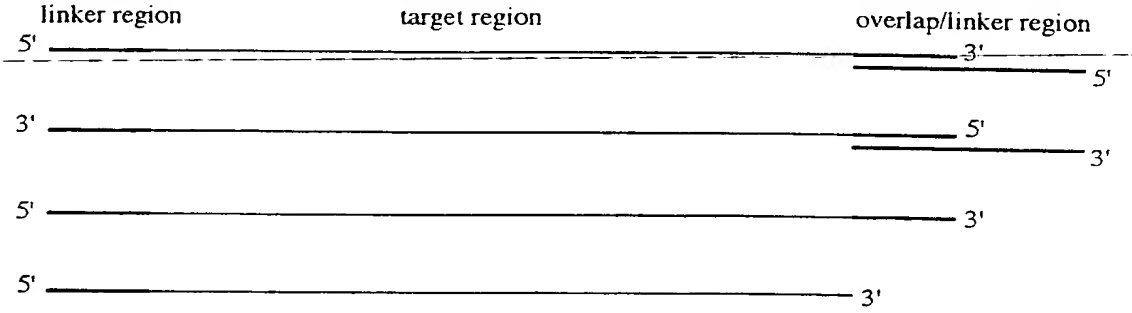
A. WRAP-PROBE Method: Component Assembly: Preferred Embodiment



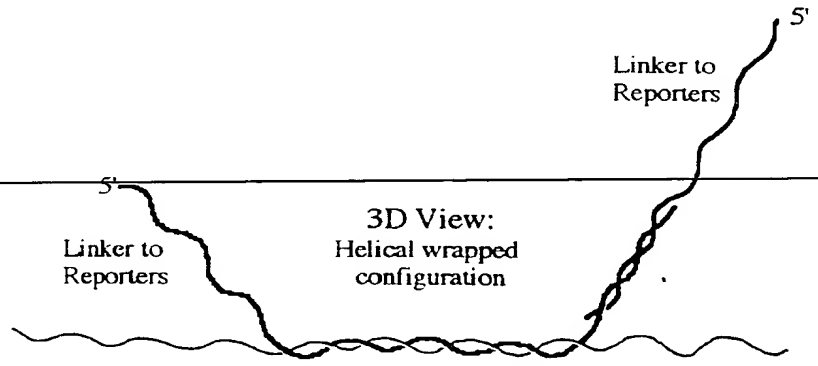

B. Alternate WRAP-PROBE Embodiments

Synthetic WRAP-PROBE made with 3' ends instead of 5' ends, or with one 5' end and one 3' end


Enzymatically made WRAP-PROBE (by PCR, Cloning, etc.) with linkers on one or both ends



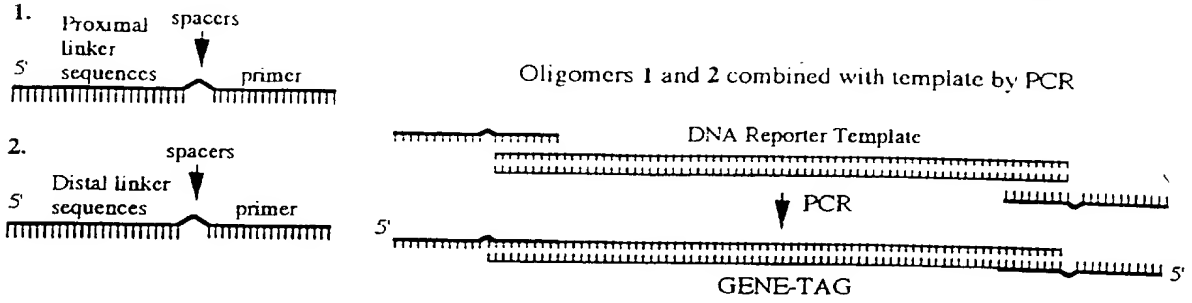

C. WRAP-PROBE Hybridized to Target Strand



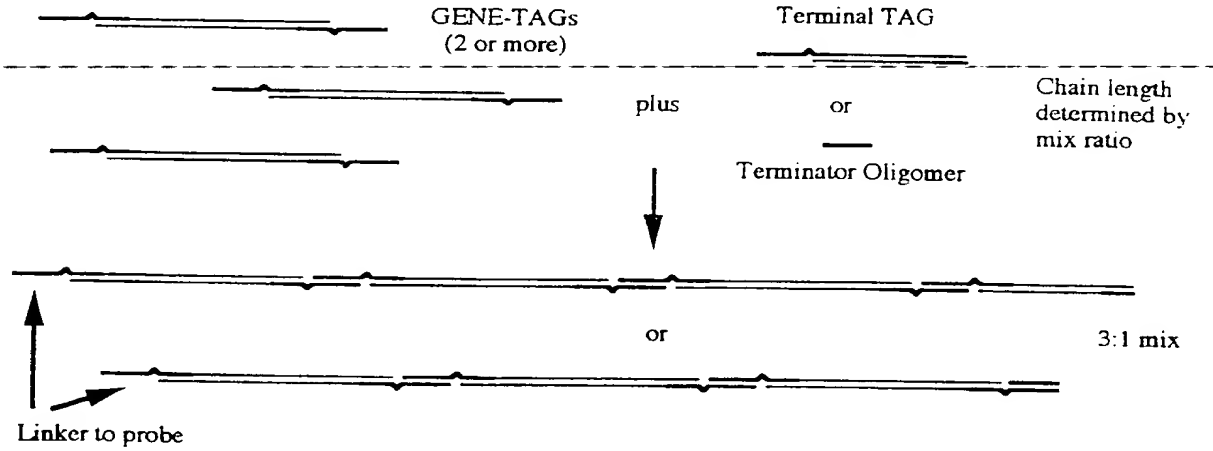

The probe wraps around the target approximately one turn per each 10 bases of sequence.

**Fig. 3**

A. Manufacture GENE-TAGs by oligomer synthesis and PCR with label or hapten




B. Assemble GENE-TAG chains by ratio mix with Terminal TAGs or Terminator Oligomers




C. Hybridize GENE-TAGs to WRAP-PROBE

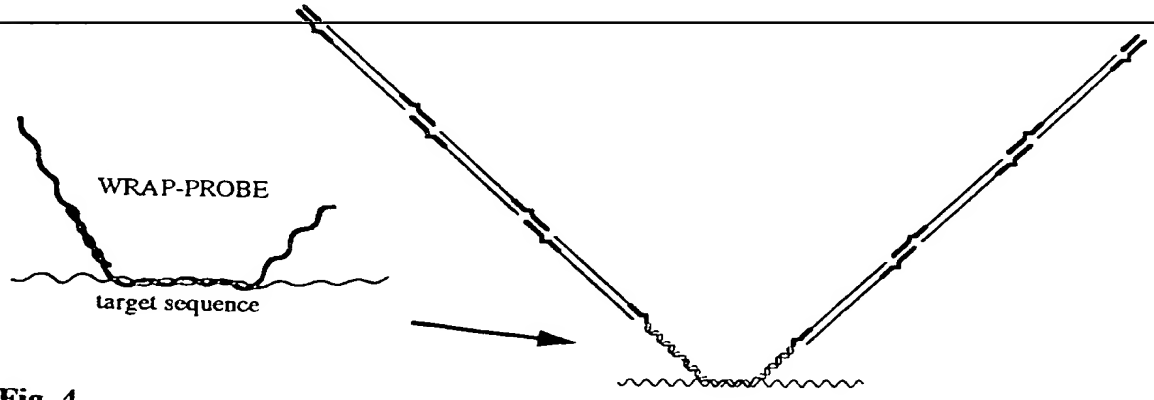
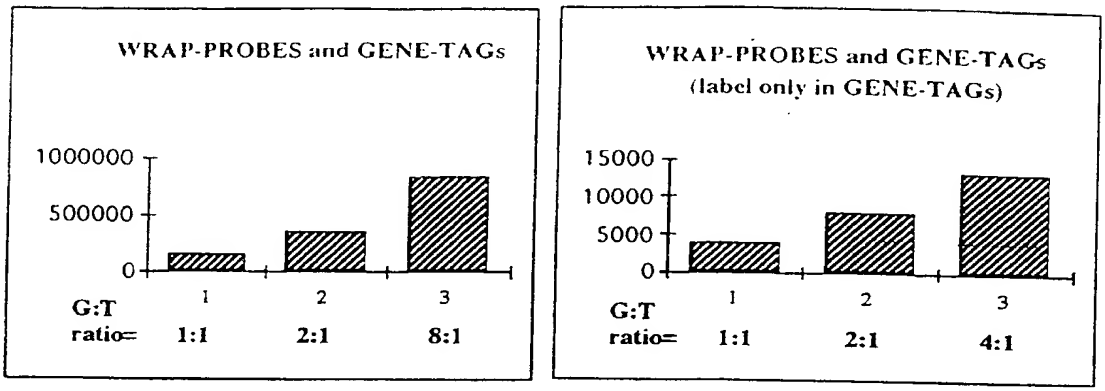



**Fig. 4**

A. Dot Blots of GENE-TAG Method with WRAP-PROBEs (Example 4)




A1. Example: Dot blots of GENE-TAGs with WRAP-PROBE-to-MTB, P32-label in all TAGs. G=Gene-TAG, T=Terminal TAG

A2. Example: Dot blots of GENE-TAGs, same as Example 2A, but no label in Terminal TAGs

B. GENE-TAGs without PCR: Synthetic Oligomer Assembly

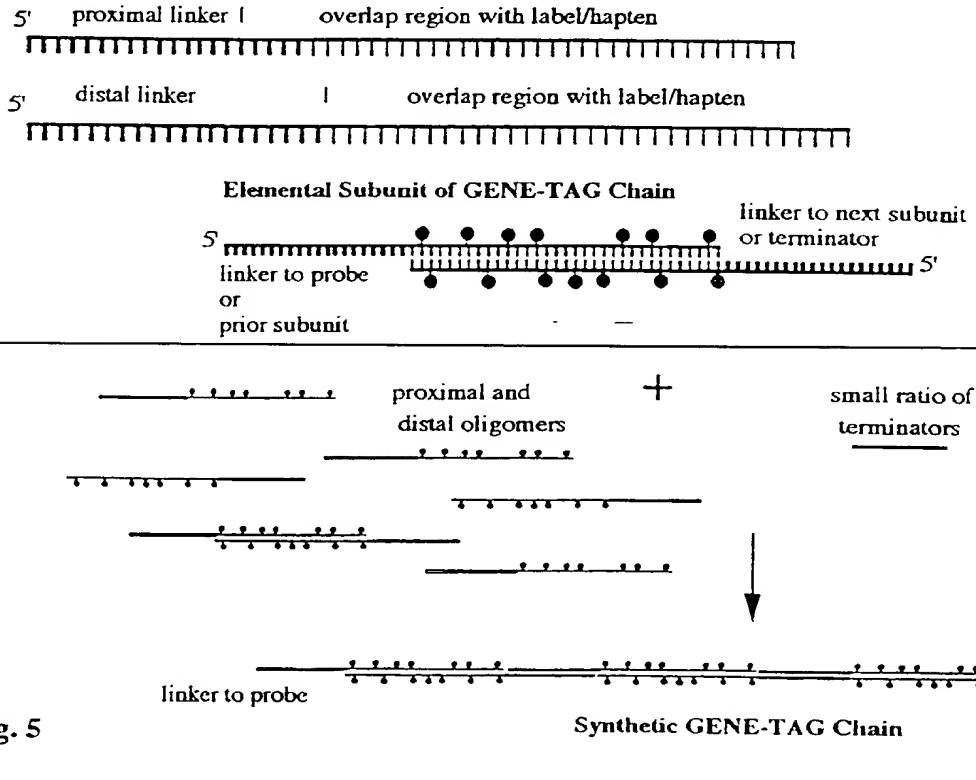
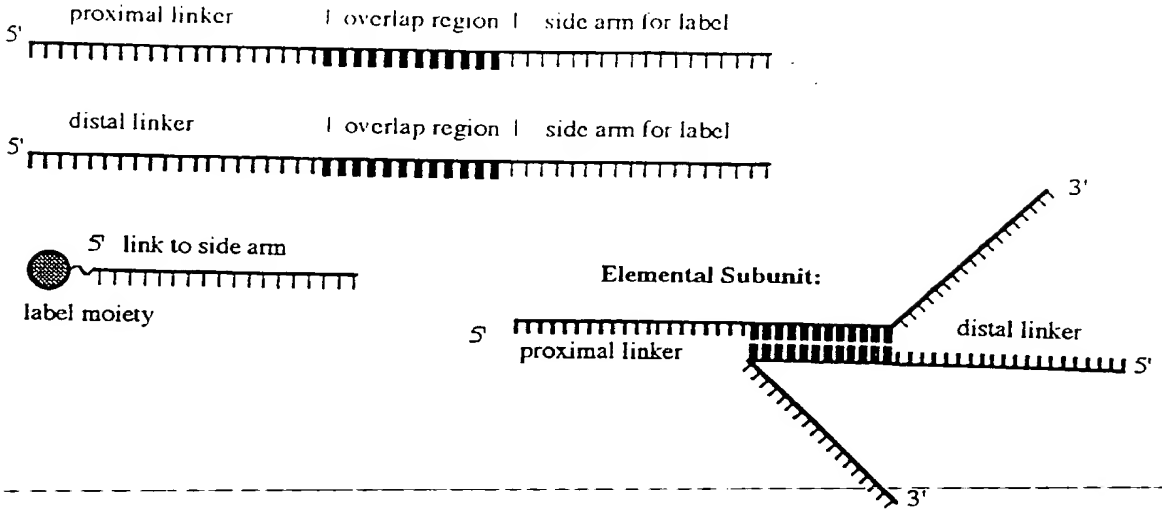



Fig. 5

A. TINKER-TAG Method: Component Design and Synthesis:




B. Component Assembly: Ratio Mix Chain Oligomers with Terminators/Label Oligomers

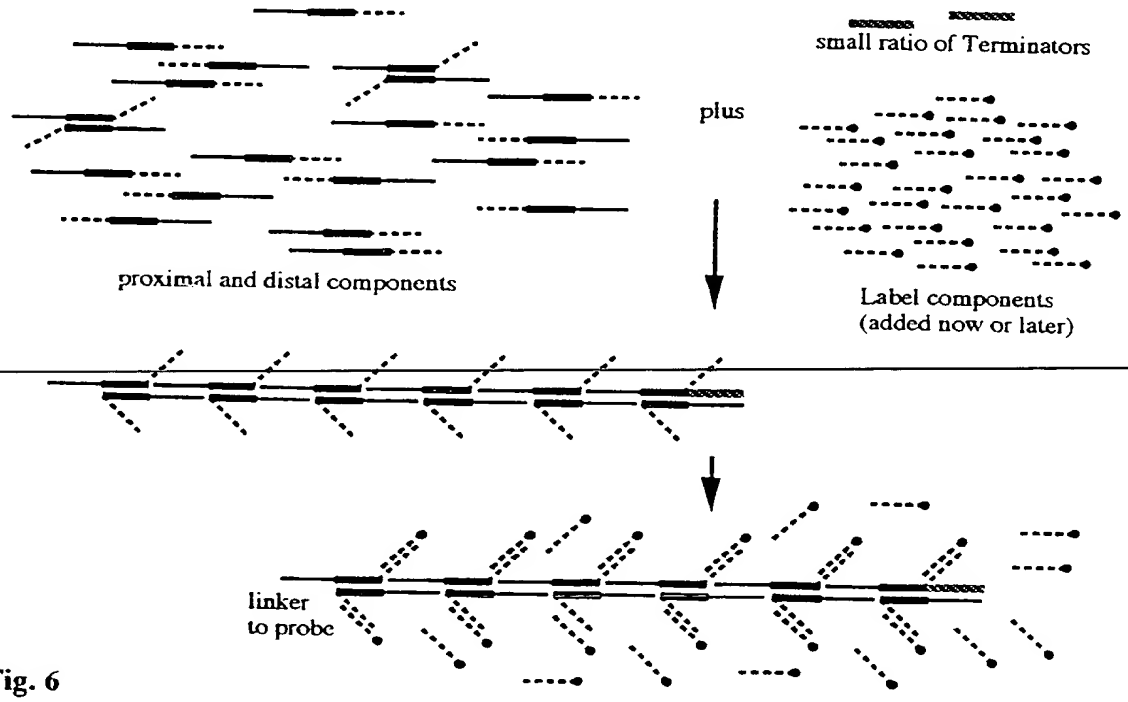
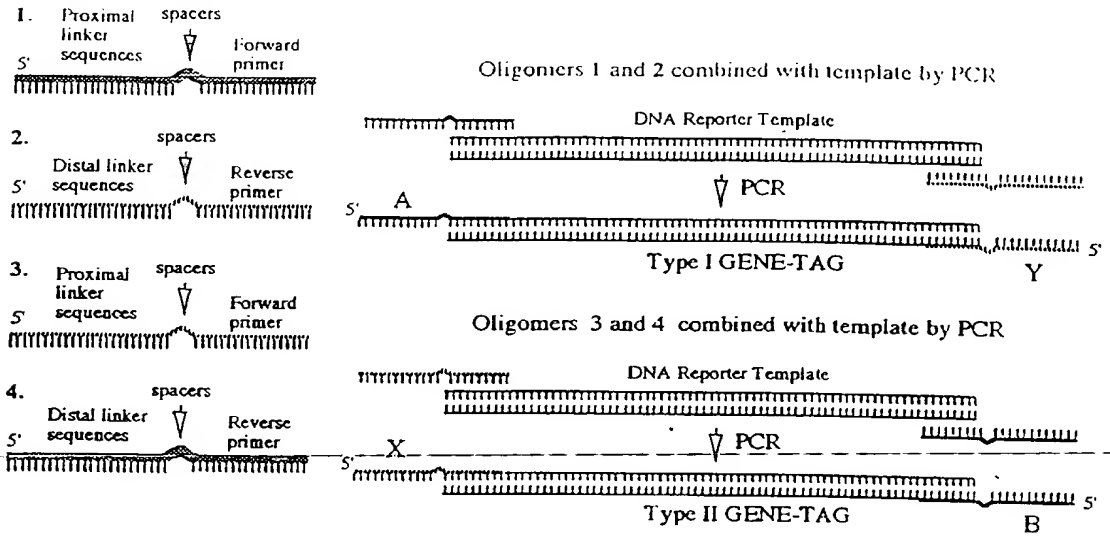



Fig. 6

A. Duo/Layered GENE-TAG Method: Construct Type I and II GENE-TAGs with alternating linkers




B. Apply Type I and Type II GENE-TAGs sequentially, washing between steps

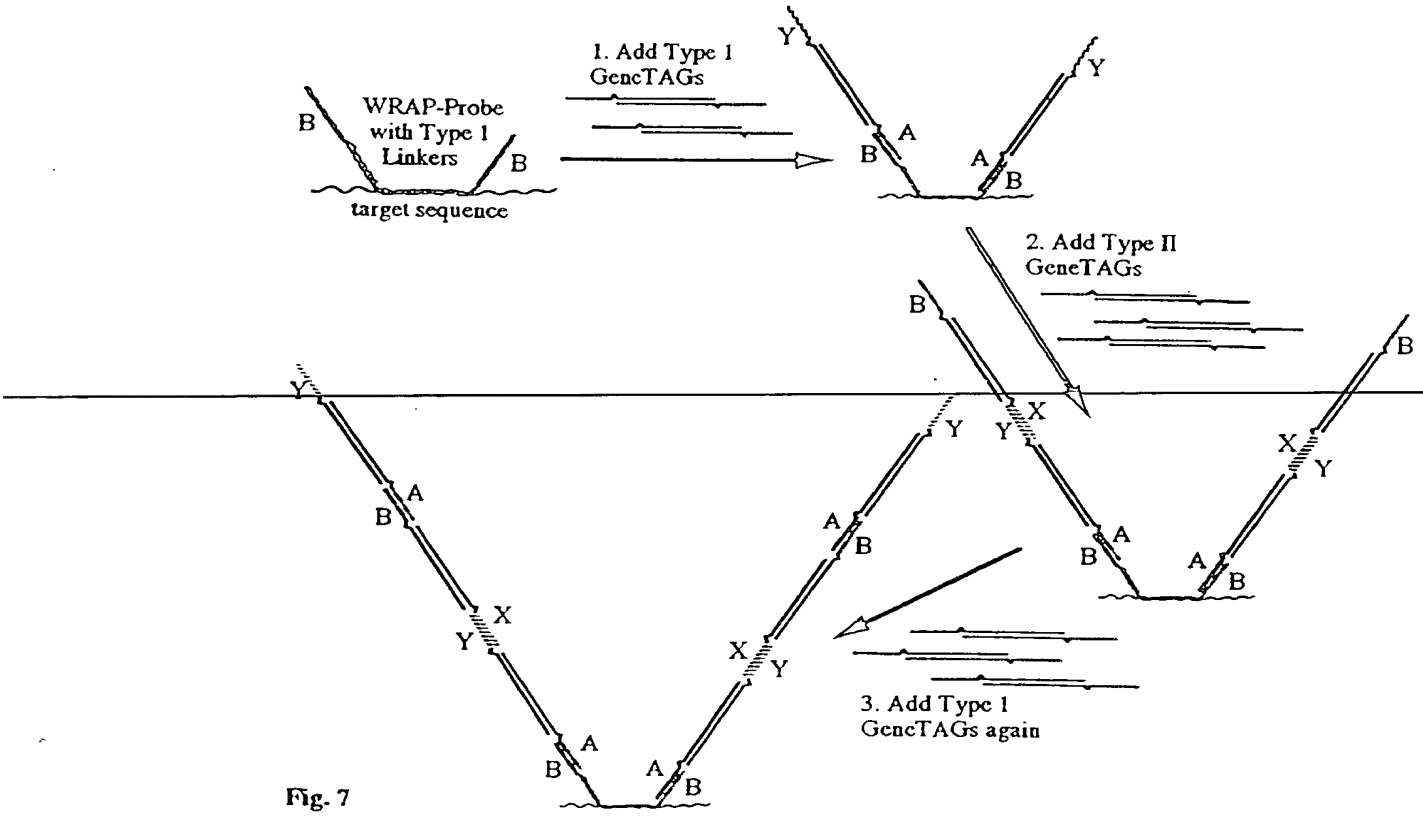
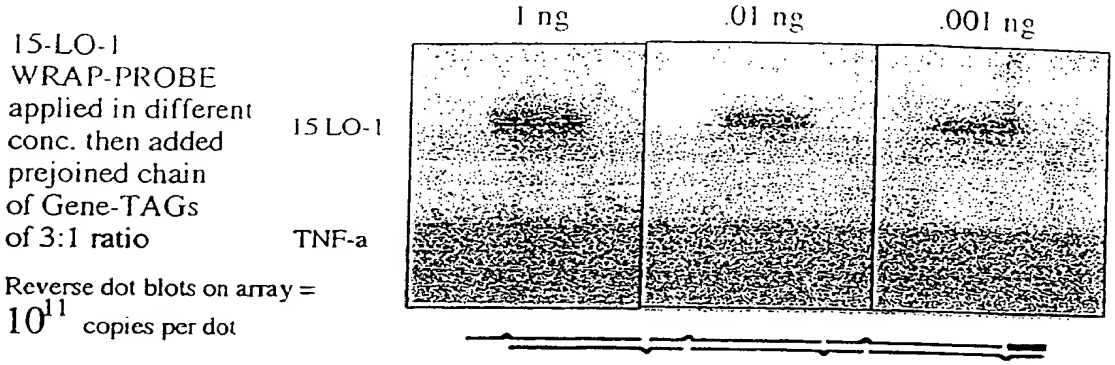



Fig. 7

A. WRAP-PROBE detection in cDNA array simulation with Chained GENE-TAGs 3:1




B. WRAP-PROBE detection in cDNA array simulation with Layered GENE-TAGs (I+II+I)

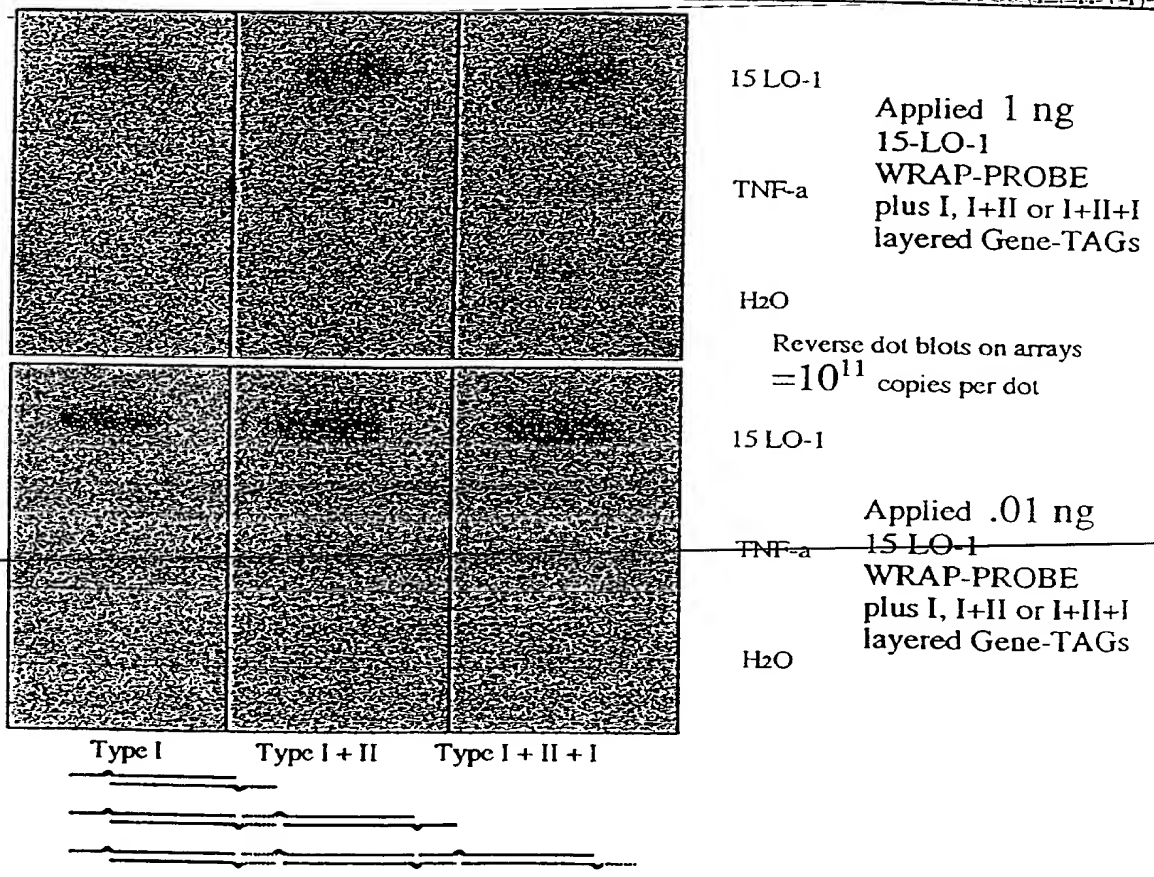
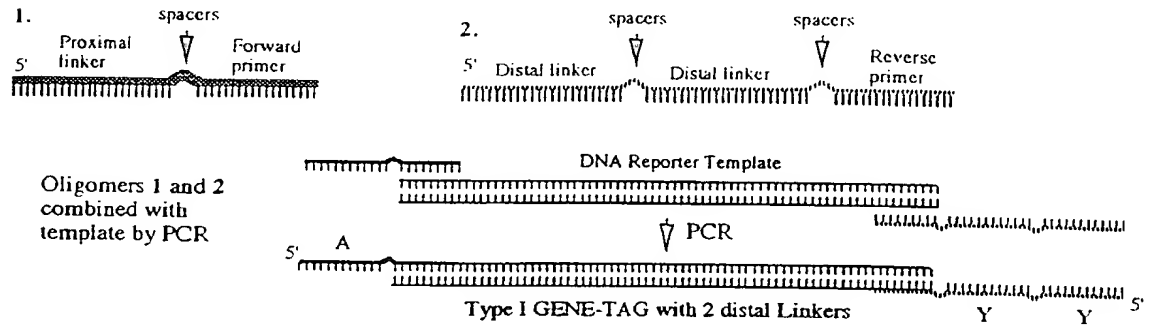
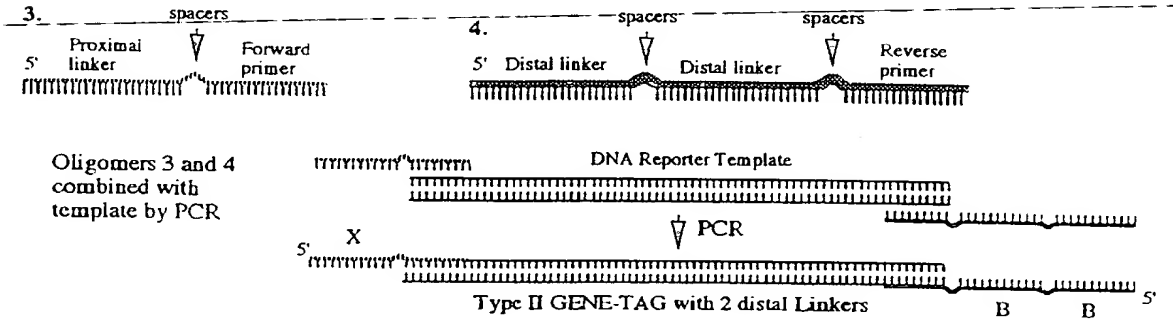



Fig. 8

## Double-Duo GENE-TAG Method: Type I and Type II plus 2 distal Linkers

A. Synthesize four GENE-TAG components and combine with reporter templates by PCR




B. Apply Double-Linker Type I and Type II GENE-TAGs in branching layers

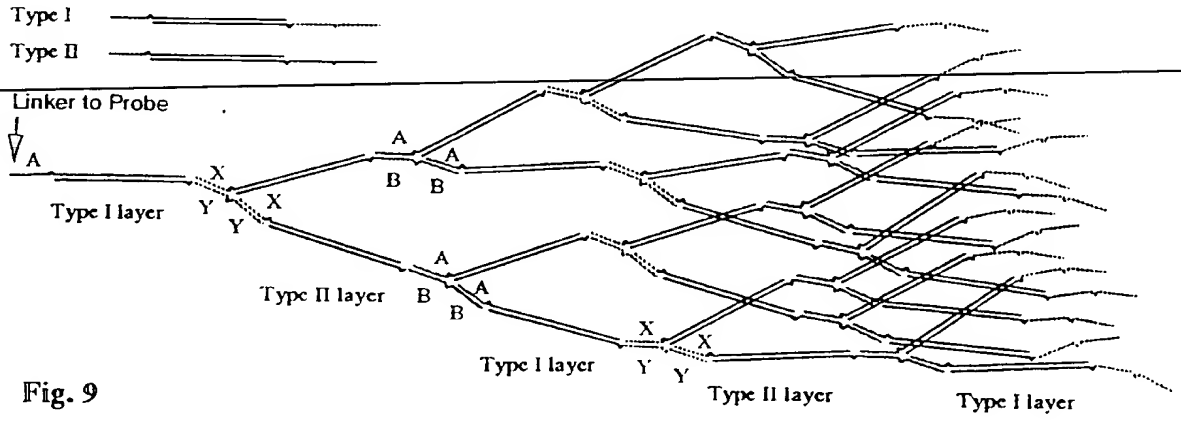
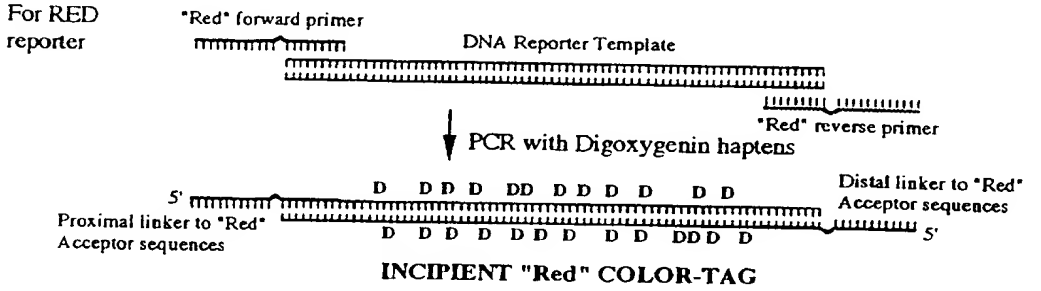


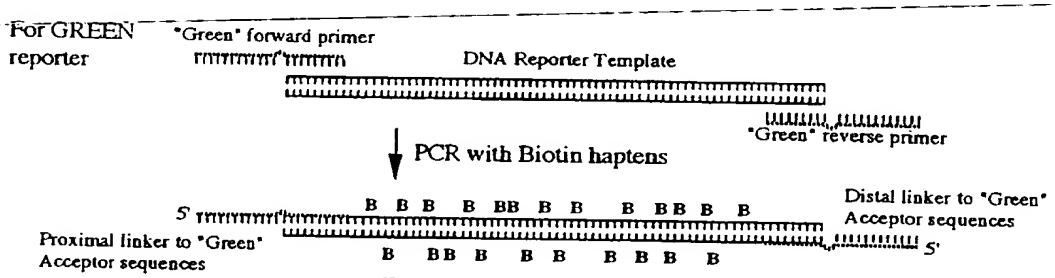

Fig. 9

COLOR-TAG Method with WRAP-PROBEs based on "Red" and "Green" COLOR-TAGs

A. Manufacture COLOR-TAG Reporters with different linkers and labeling




INCIPIENT "Red" COLOR-TAG




INCIPIENT "Green" COLOR-TAG

B. Construction of three WRAP-PROBES for COLOR-TAG Application:

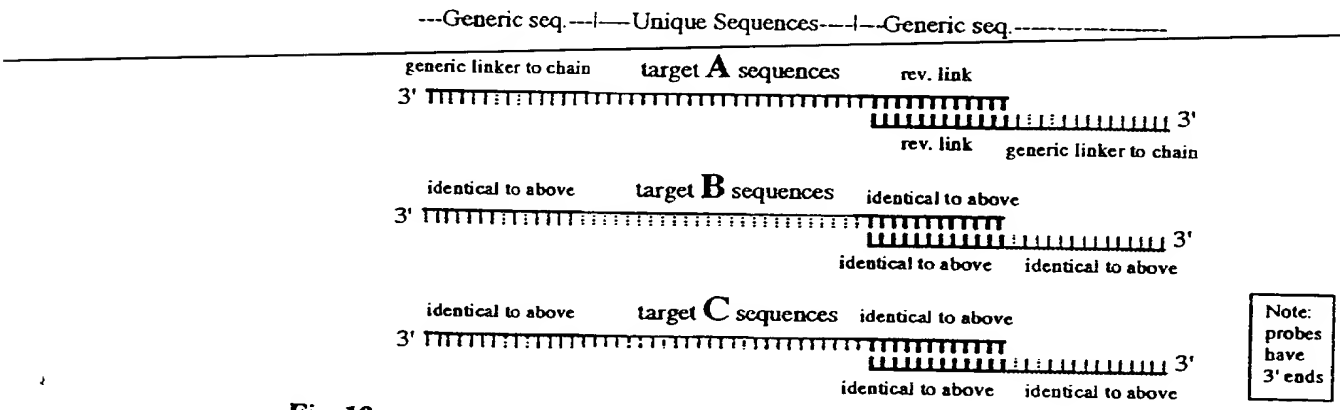
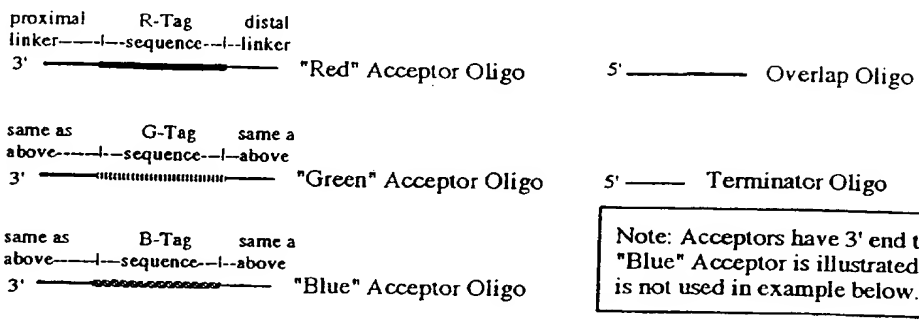


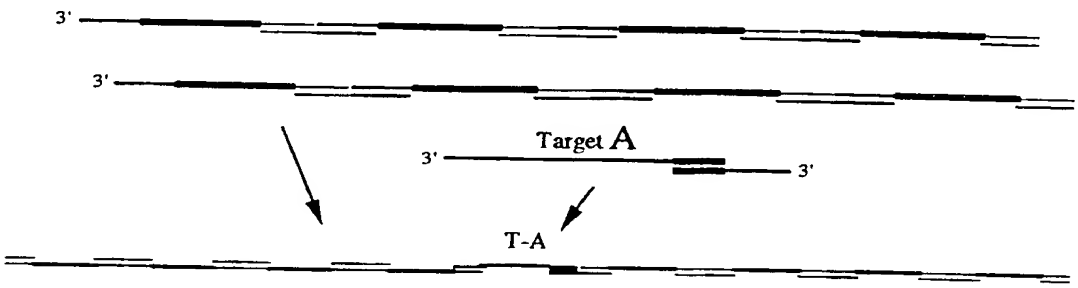

Fig. 10

COLOR-TAG Method (Part 2):

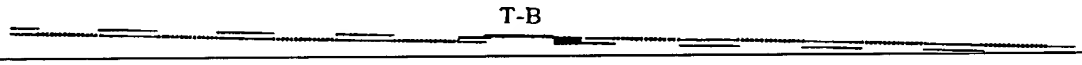
A. Synthesize COLOR-LINKER Chain Components:




B. Assemble "Red" COLOR-LINKER chain, mix on 2:1 basis with Target A Probe (eg. ABR)




C. Similarly Assemble "Green" COLOR-LINKER chain with Target B Probe (eg. D17S379)




D. Assemble 1/2 "Red" 1/2 "Green" COLOR-LINKER chain with Target C Probe (eg. CHR-12)

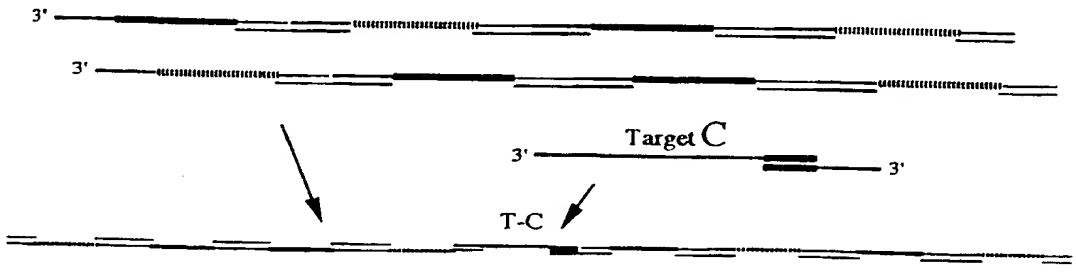
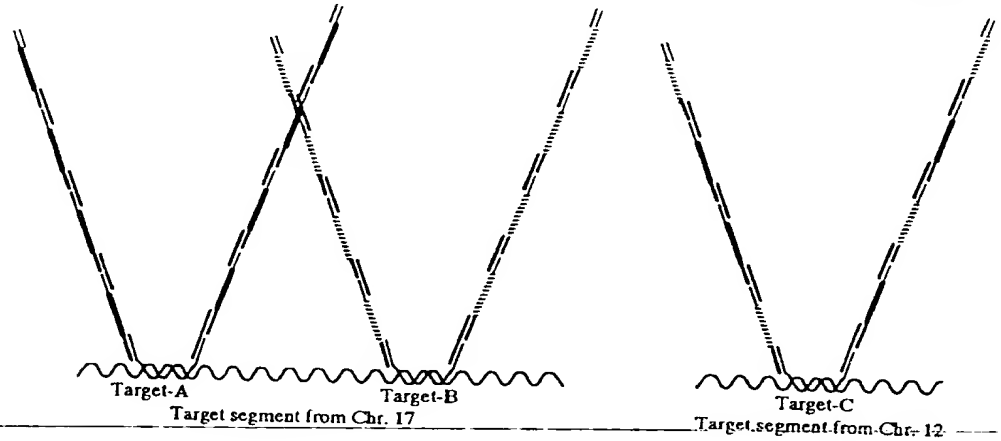


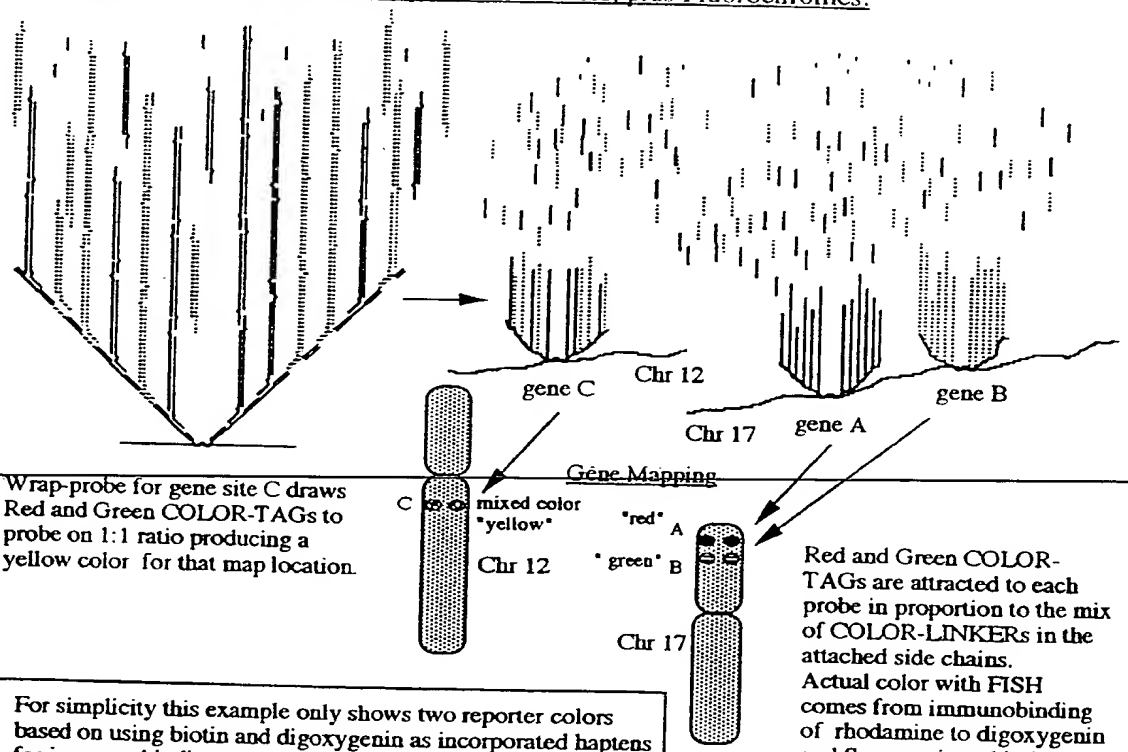

Fig. 11

COLOR-TAG Method (Part 3):

A. Apply WRAP-PROBEs with probe specific COLOR-LINKER chains to Targets




B. Apply set of COLOR-TAGs, plus Terminators, plus Fluorochromes:




Wrap-probe for gene site C draws Red and Green COLOR-TAGs to probe on 1:1 ratio producing a yellow color for that map location.

Red and Green COLOR-TAGs are attracted to each probe in proportion to the mix of COLOR-LINKERs in the attached side chains. Actual color with FISH comes from immunobinding of rhodamine to digoxygenin and fluorescein to biotin.

For simplicity this example only shows two reporter colors based on using biotin and digoxygenin as incorporated haptens for immuno-binding of two fluorochromes such as FITC and rhodamine. Three color mixing can be easily achieved with alternate haptens and immuno-bound fluorochromes or with incorporation of bases that have fluorchromes directly attached.

**Fig. 12**

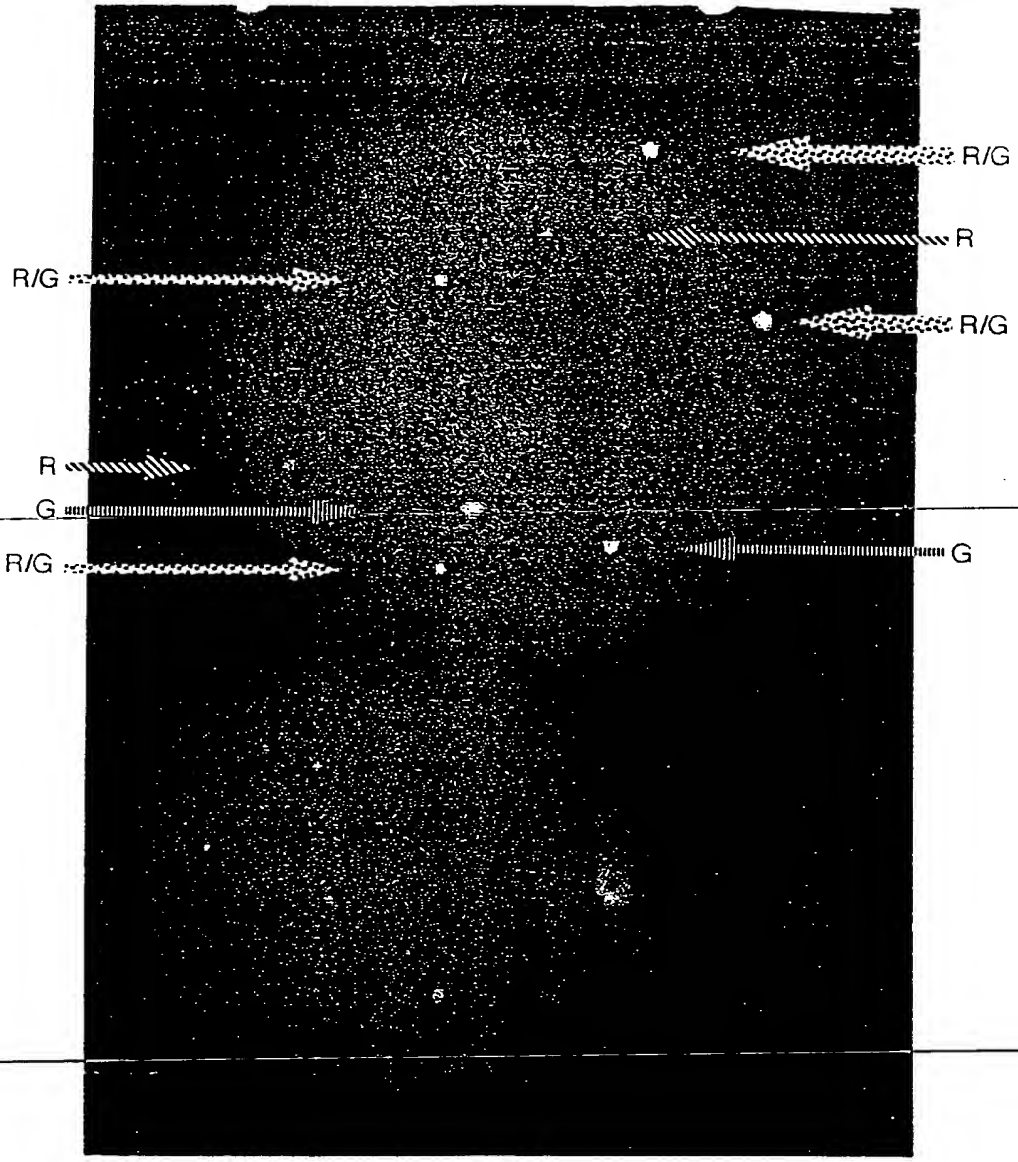



Photo image of FISH detection with four WRAP-PROBEs with attached chains of either Red COLOR-TAGs, Green COLOR-TAGs or mixed Red and Green COLOR-TAGs. In nuclei, four pairs of detected dots are expected. For this gray tone copy of the color image, colors and sizes indicated by arrow patterns

Chr. 12 short repetitive site, mixed red/green R/G:

15-LO, green: G: ABR, red: R:

Marker, mixed R/G: All four WRAP-Probes have a target of 30 bp or less

Color filtering provides a clear spectral discrimination of the detection of a specific color and the presence of mixed color

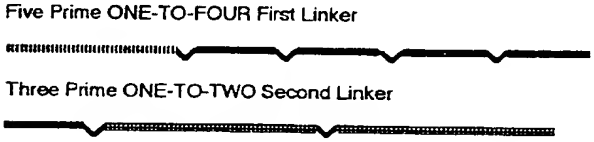
**Fig. 13**

A. Multi-LINKER Method: Elemental form as Single Synthetic Oligonucleotide

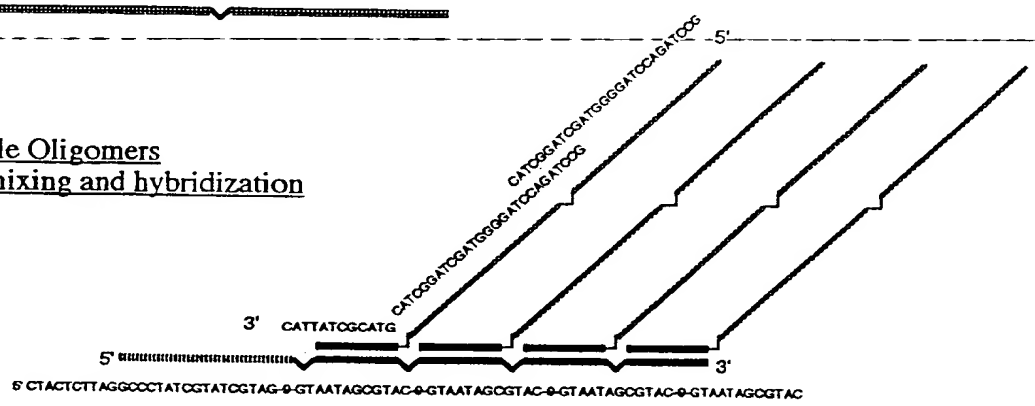

Generally a Proximal probe-specific Linker (5' or 3') plus two or more Distal reporter-specific Linkers

B. Preferred Two-Part Multi-LINKER embodiment for binding 8 GENE-TAGs

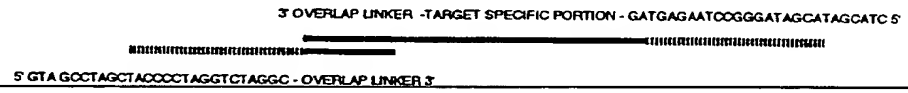
Synthesize Oligomers:




Assemble Oligomers
by 4:1 mixing and hybridization




Assemble WRAP-Probe by hybridization




Assemble and crosslink probe and Multi-LINKER units

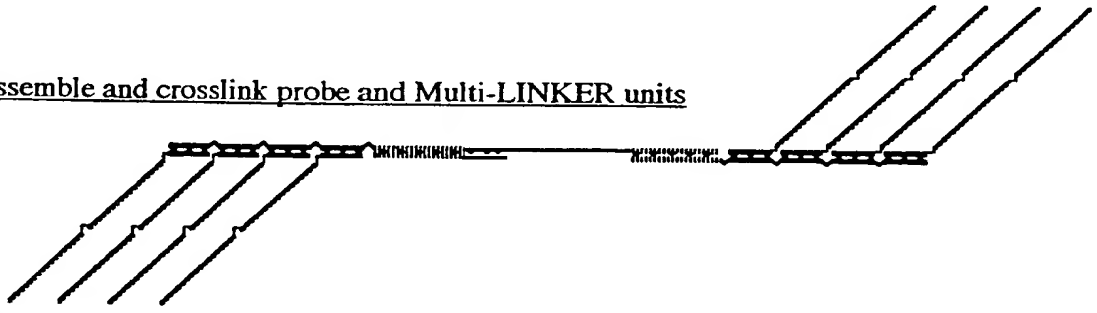


Fig. 14

A. Multi-LINKER Method: Preferred Three-Part embodiment for binding a multiplicity of short oligomers that have 5' prejoined labeling agents

Synthesize Multi-LINKER "Red" set and oligo label units with "Red" fluor (eg. Cy5)

5' CTACTCTTAGGCCCTATCGTATCGTAG-9-GTAATAGCGTAC-9-GTAATAGCGTAC-9-GTAATAGCGTAC-9-GTAATAGCGTAC

5' CTAGGTAGCTAG-9-CTAGGTAGCTAG-9-CTAGGTAGCTAG-9-CTAGGTAGCTAG-9-GTACGCTATTAC

5' CTAGCTACCTAG-99-GTACGTAACTAG-99-GTACGTAACTAG-99-GTACGTAACTAG-99-GTACGTAACTAG

"Red" fluor such as Cy5 5' cy5–CTAGTTACGTAC

Synthesize Multi-LINKER "Green" set and oligo label units with "Green" fluor (eg. Cy3)

5' GCCTAGACCTAGGGGTAGCTAGGCTAC-9-CTACCTATCTAC-9-CTACCTATCTAC-9-CTACCTATCTAC-9-CTACCTATCTAC

5' CTAGGTAGCTAG-9-CTAGGTAGCTAG-9-CTAGGTAGCTAG-9-CTAGGTAGCTAG-9-GTACGCTATTAC <---same as second oligo in "Red" set above

5' CTAGCTACCTAG-99-CTATCTAGTACG-99-CTATCTAGTACG-99-CTATCTAGTACG-99-CTATCTAGTACG

"Green" fluor such as Cy3 5' cy3–CGTACTAGATAG

B. Assembly by hybridization and crosslinking

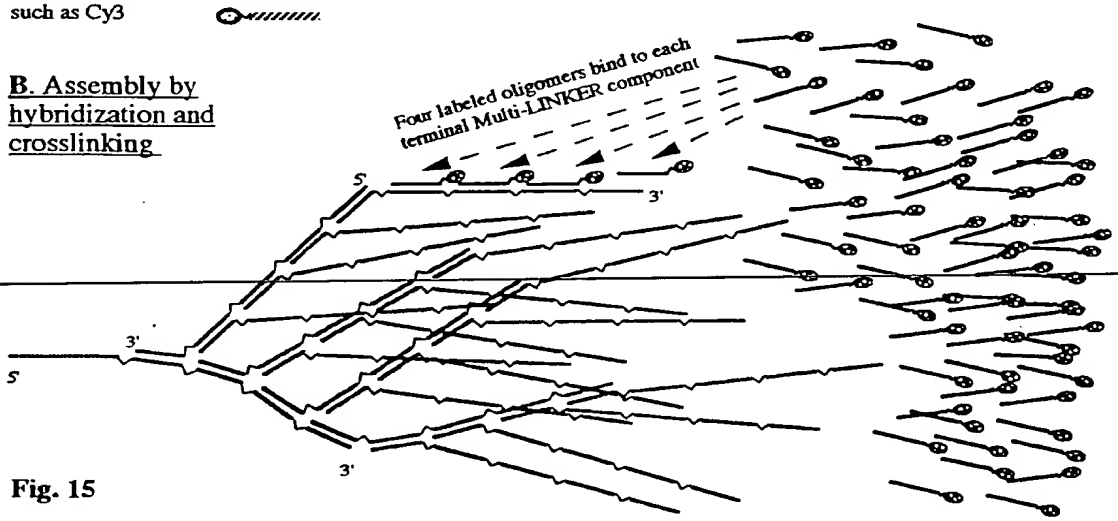



Fig. 15

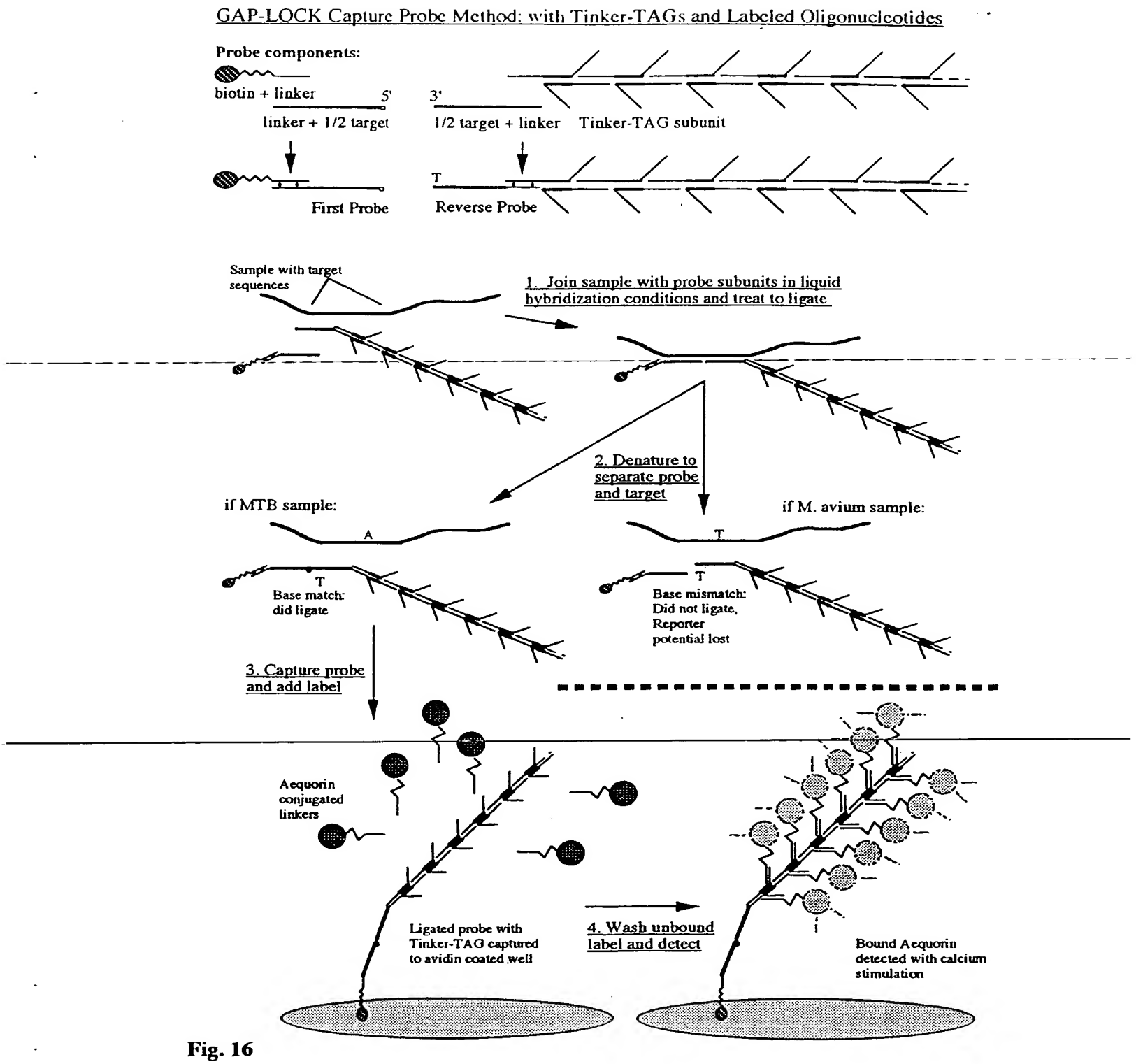
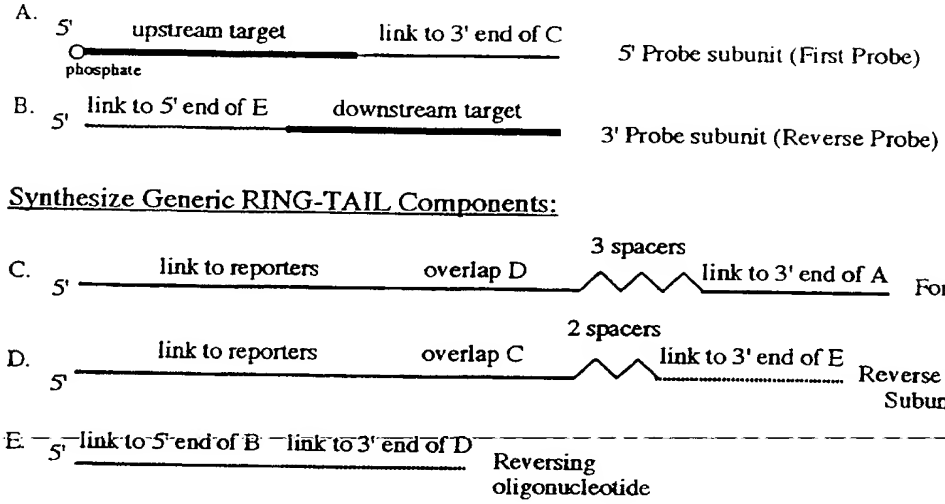

GAP-LOCK Capture Probe Method: with Tinker-TAGs and Labeled Oligonucleotides
Probe components:
biotin + linker
5'
linker + 1/2 target
3'
1/2 target + linker
Tinker-TAG subunit
First Probe
T
Reverse Probe
Sample with target sequences
1. Join sample with probe subunits in liquid hybridization conditions and treat to ligate
2. Denature to separate probe and target
if MTB sample:
A
T
Base match: did ligate
if M. avium sample:
T
T
Base mismatch: Did not ligate, Reporter potential lost
3. Capture probe and add label
Aequorin conjugated linkers
Ligated probe with Tinker-TAG captured to avidin coated well
4. Wash unbound label and detect
Bound Aequorin detected with calcium stimulation

**Fig. 16**

RING-LOCK PROBE METHOD:

A. Synthesize Target Specific GAP-LOCK Components:



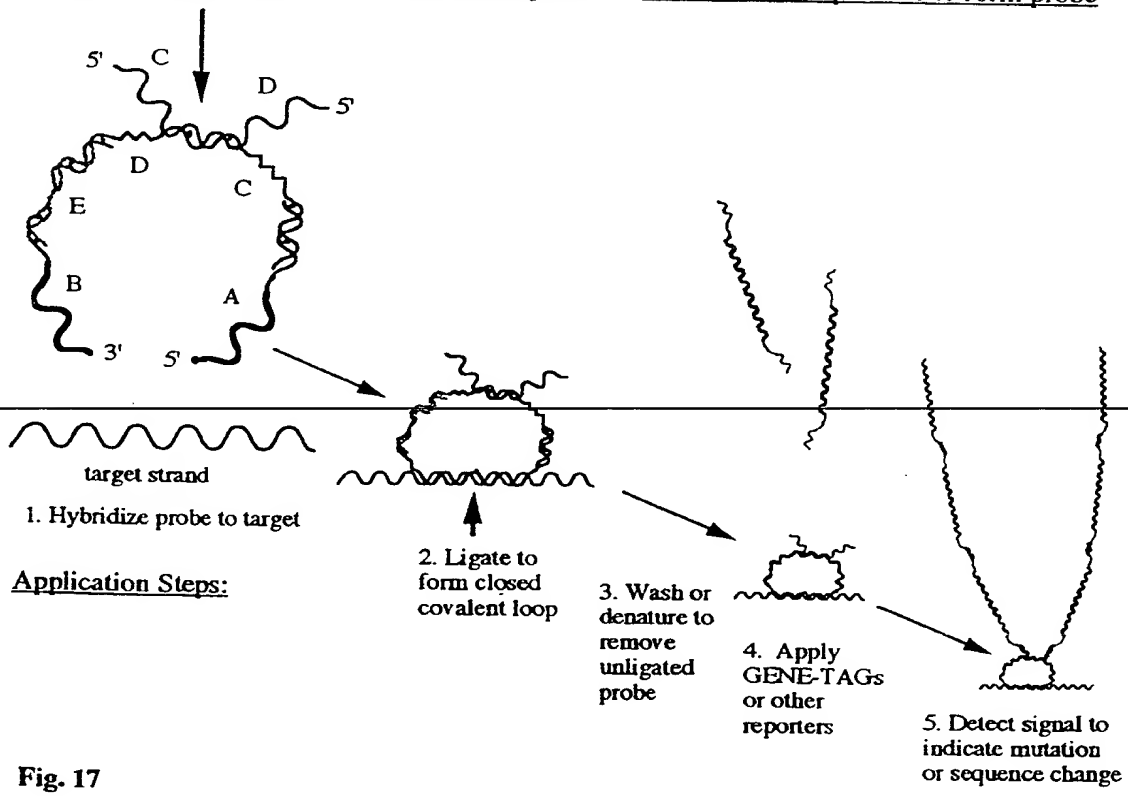

Synthesize Generic RING-TAIL Components:







B. Assembly: Hybridize A B C D and E together and crosslink components to form probe




Application Steps:

Fig. 17

WRAP-LOCK PROBE METHOD:

A. Synthesize Target Specific WRAP-PROBE Component:

A. 5' link to 5' end of D — target region — link to 3' end of B

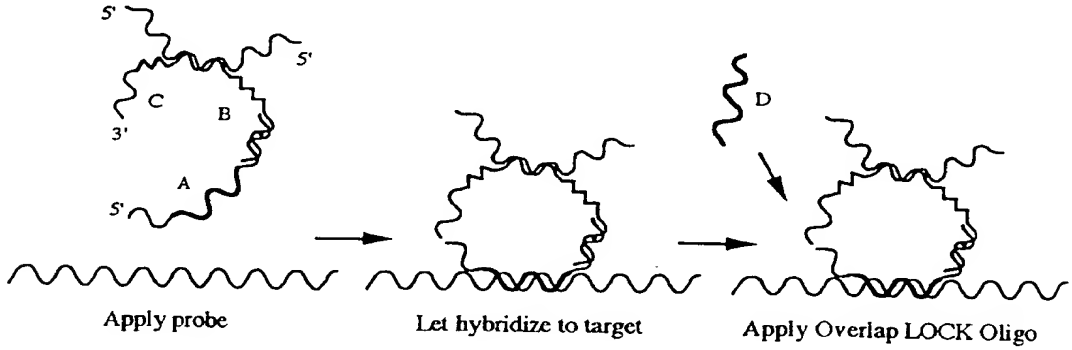
Synthesize Generic RING-TAIL Components:

B. 5' link to reporters — overlap C — 3 spacers — link to 3' end of A — Forward Ring Subunit

C. 5' link to reporters — overlap B — 2 spacers — link to 3' end of D — Reverse Ring Subunit

D. 5' link to 5' end of A — link to 3' end of C — Lock Component

B. Assemble and crosslink four probe components and Apply to target in steps below:



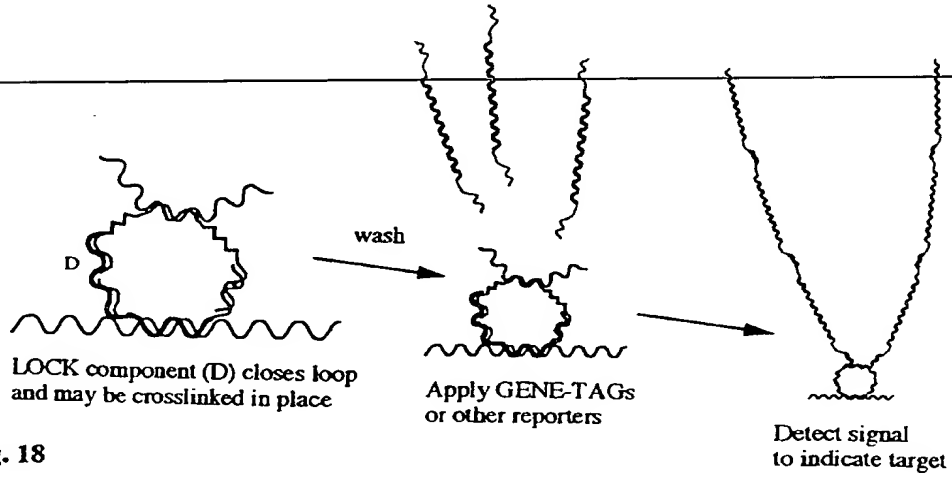
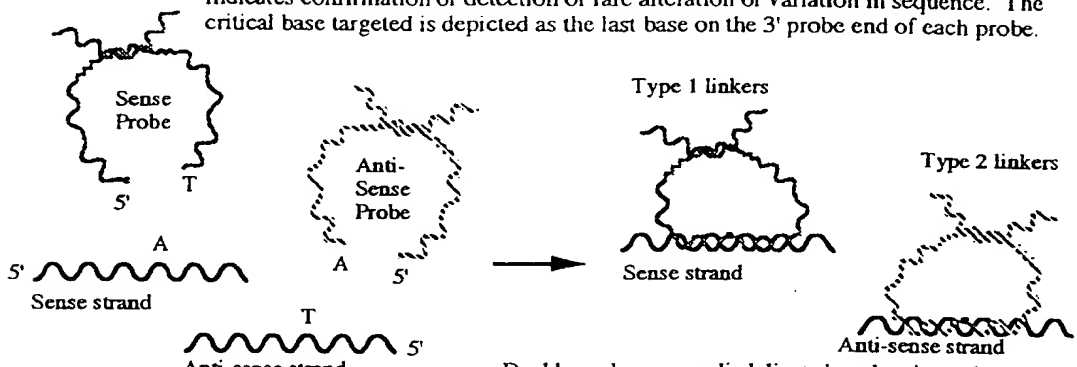




Fig. 18

DOUBLE-LOCK Probe Method: Employs same probe design as RING-LOCK Probe:

A.

Components based on one probe targeting the sense strand alteration, the other targeting the complementary anti-sense strand alteration. Two hits of two color or signal type indicates confirmation of detection of rare alteration or variation in sequence. The critical base targeted is depicted as the last base on the 3' probe end of each probe.




Double probes are applied, ligated, and stringently washed or denatured to remove unligated sense or antisense probes

B.

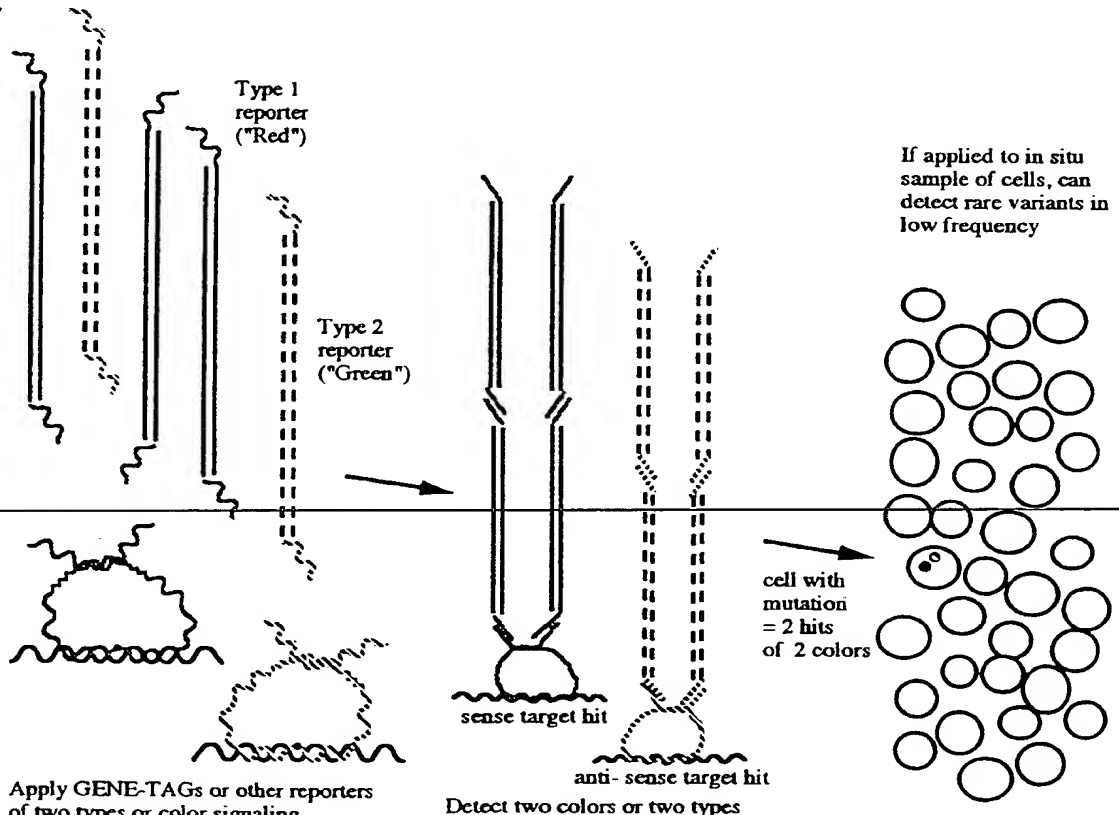



Fig. 19

GOLD-TAG Method: Gold plus Silver TINKER-TAGs applied to WRAP-PROBEs

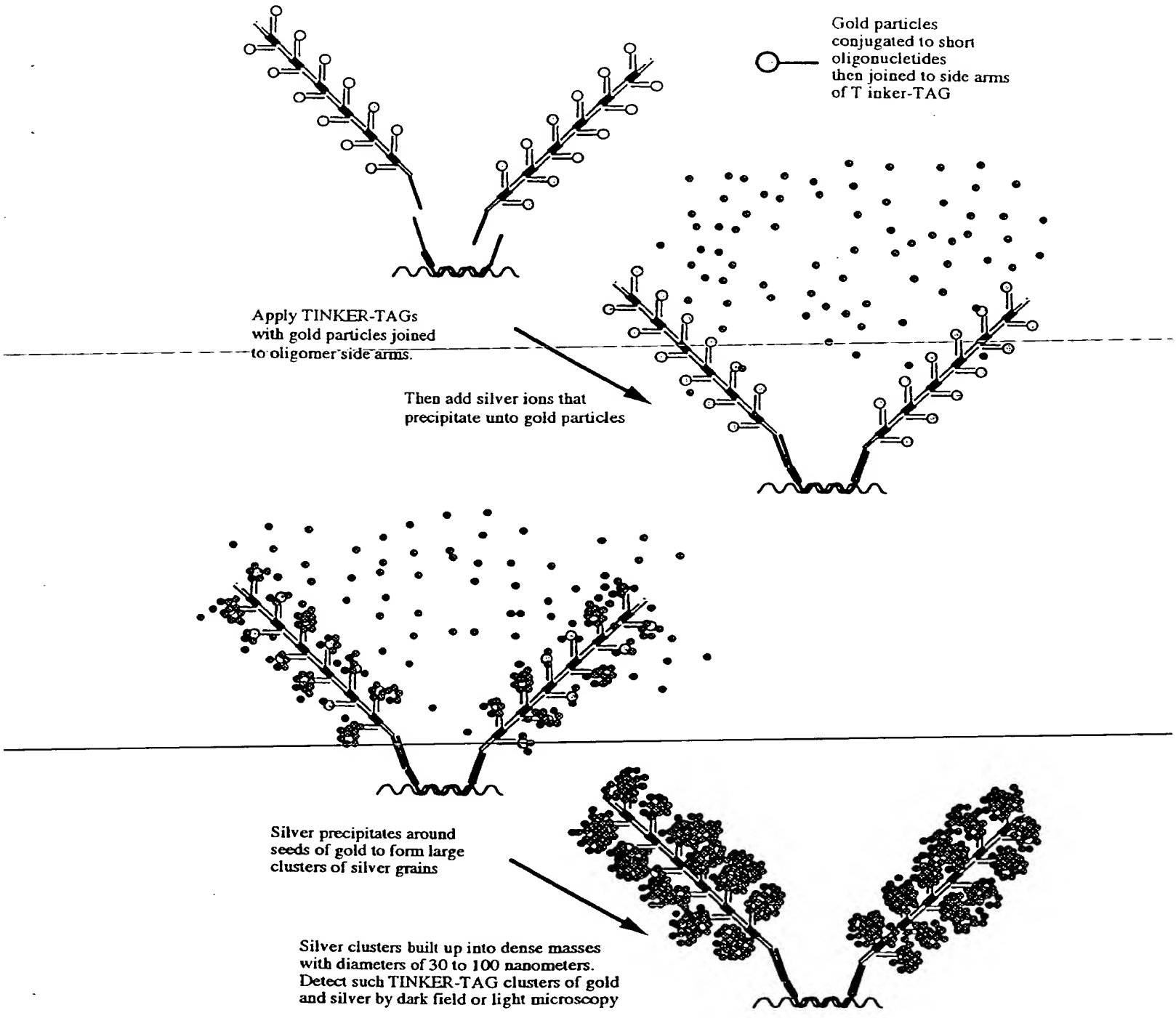
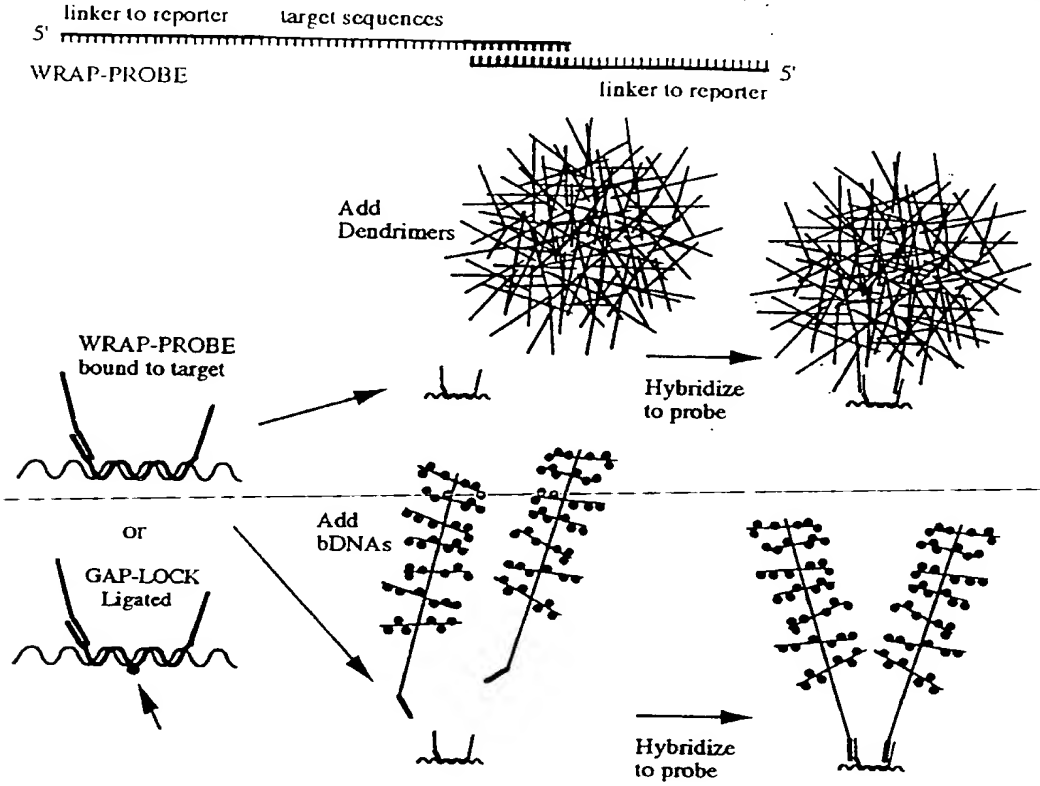
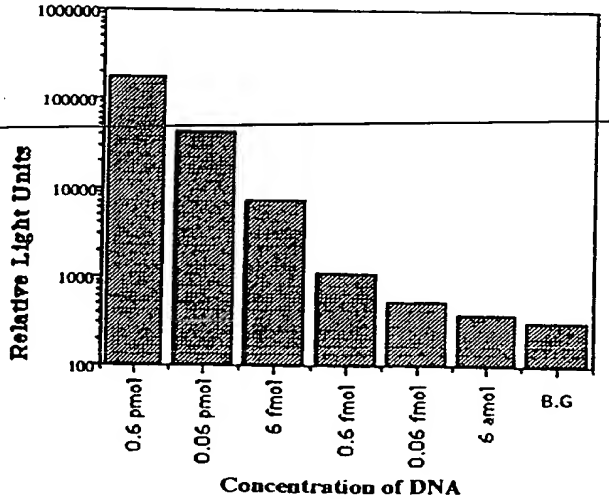



**Fig. 20**

A. Apply WRAP-PROBEs (or GAP-LOCK) with Dendrimers and bDNA signaling products:




B. Aequorin Detection of MTB DNA using GAP-LOCK First Probe: (Example 9)




luminometer readout of aequorin labeled First Probe detecting a series of dilutions of MTB cDNA. B.G= background detection of an unrelated control DNA at .6 pmol.

Fig. 21